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PREVALENCE AND DIVERSITY OF ECTOPARASITES (TICKS AND FLEAS) IN BLACK RATS (RATTUS RATTUS) AROUND GRAIN STORAGE FACILITIES IN PUNJAB, PAKISTAN

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Abstract

The presence of food and shelter in grain storage facilities may provide the necessary environment to support both the rodents and their ectoparasites and is associated with the presence of black rats (Rattus rattus). Ticks and fleas that are found on rodents are of veterinary and public health significance as they may be vectors of infectious agents. The prevalence and diversity of the ticks and fleas associated with rodents around grain storage areas in Punjab however are still not known. The objectives of this study were to estimate the prevalence, diversity and intensity of infestation of ticks and fleas taken from R. rattus in the vicinity of selected grain storage facilities. Standardised collection and identification of ectoparasites were performed on rodents. Preserved specimens collected were identified morphologically following the appropriate taxonomic keys. The prevalence of ectoparasites was estimated as the percentage of rodents examined with at least one ectoparasite and mean intensity and mean abundance were used to indicate the intensity of ectoparasite infestation. When possible, differences in infestation patterns were assessed based on (a) sampling location, (b) season, (c) host

characteristics, and (d) storage-facility conditions. This study framework is used to document the ectoparasite fauna of black rats in grain storage environments and potential ecological factors that relate to infestation. Results can contribute to integrated rodent management, environmental sanitation and selective ectoparasite surveillance in agricultural production environments. Also, taxonomic documentation can offer baseline data for future investigations that include molecular identification and pathogen screening. This will help clarify epidemiological implications of rodent ectoparasites and will aid in evidence based management for rodent ectoparasites in Pakistan. The study also emphasizes the need to regularly monitor the vicinity of grain storage to better manage pests and mitigate potential risks posed by grain storage–rodent interaction with ectoparasites.

INTRODUCTION

The black rat (Rattus rattus) is a commensal rat that is found all over the world, and is often encountered in agricultural areas, in food-storage facilities, in warehouses, and in human settlements. They are adaptable, prolific, and are in close association with stored products, and act as hosts to ectoparasites (Ho et al., 2021; Islam et al., 2021). The relevance of flea and tick associated with rodents to veterinary and public health lies in their ability to participate in the transmission of certain pathogens (El Hamzaoui et al., 2020; Obiegala et al., 2021). Adopting a systematic review of rodents is associated with the presence of a great variety of ectoparasitic fauna, and significant interregional variation (Ho et al., 2021). This research highlights the importance of surveillance and identification of ectoparasites associated with rodents in a region-specific manner. Food resources, shelter, and nesting opportunities may allow persistent rodent populations to exist in grain storage facilities. However, a number of factors, such as host characteristics, environmental conditions, habitat, and parasite life cycles all play a role in ectoparasite infestation (Mohammadi et al., 2020). Studies conducted by Enscore et al. (2020) have shown the significance of the host communities for the distribution of fleas, and how invasive black rats affect the abundance of native small mammals. Habitat conditions have also been studied in rodents across habitats with varying anthropogenic disturbance, relating to changes in diversity of ectoparasites (Gil-Fernández et al., 2024). Infestation patterns may also be affected by seasonal changes as various groups of ectoparasites may be more or less responsive to environmental factors (Desoky, 2020). Fleas and ticks have different host associations, development, and environmental needs. The distribution can consequently, differ from place to place and from time to time (Islam et al., 2021). Various ectoparasite taxa have been reported in India with rodents and shrews such as R. rattus (Paulraj et al., 2022). Variability in the distribution of ectoparasites of rodents has also been reported from various locations in the Middle East (Soe et al., 2026). These lead to the need for morphological identification and standard sampling to estimate prevalence, abundance and the diversity of ectoparasites. In addition, some ectoparasites are associated with pathogens of human and veterinary importance, and some of these species are linked to rodents, potentially giving epidemiological importance to these parasites. The study conducted in Brazil highlighted the importance of studying host–parasite relationships in habitats with a synanthropic nature (Goncalves et al., 2020). However, the presence of ticks or fleas on rats does not indicate whether or not they are carrying or transmitting pathogens. Molecular testing is required, if warranted, along with appropriate identification to determine the epidemiological significance of these associations. Although this is an international evidence, the prevalence, the taxonomic diversity and the infestation intensity of ticks and fleas on black rats around grain storage facilities in Punjab, Pakistan is not well characterized. The objectives of the present study are, therefore, to estimate the prevalence of tick and flea infestation in R. rattus, to characterize the most probable taxonomic level of the ectoparasites found, to determine their intensity and abundance, and to examine the variation in terms of host characteristics, sampling site, season, and storage-facility conditions. The results could help to enhance grain storage hygiene, rodent control, and future studies on the epidemiological role of ectoparasites associated with rodents.

2. Materials and Methods

2.1. Study Area and Study Period

This study aimed to assess the prevalence and diversity of ticks and fleas of black rats (Rattus rattus) in grain storage areas of Punjab, Pakistan. Based on the objectives of the study, the selected grain storage facilities were to be included in representative districts of Punjab which include commercial grain storehouses, grain markets, agricultural grain storehouses and facilities associated with animal-feed storage. The sampling locations were to be chosen based on ease of access to the locations, evidence of rodent activity, facility type, and facility managers' willingness to allow access for sampling. The geographic location of each participating facility was to be determined by taking advantage of its location using a global positioning system (GPS) device for comparison of spatial location between sampling sites. The sampling period was designed to include a variety of seasonal conditions to assess possible temporal fluctuations of ectoparasite infestation. Climatic data, such as temperature, relative humidity and rainfall (if available) at the district level is to be sourced from relevant meteorological records. These environmental factors were related to observed patterns of infestation.

2.2. Study Design and Sampling Strategy

A cross sectional, multi-site observational study design was used to examine ectoparasite parasitism of black rats in the environment of grain storage. Some facilities were selected within the districts to ensure the representation of a variety of storage conditions and decrease the reliance on individual facilities. The primary sampling unit was the individual rat captured, and the grain storage facility was the higher level sampling unit to assess site related variation. A standardized sampling record was developed for each rat, with a unique identification code, the sampling site, when it was captured, and host characteristics. The sample size was to be determined based on the expected prevalence of ectoparasite infestation, a prespecified level of confidence and an acceptable margin of error, plus an allowance for clustering within facilities and possible incomplete observations. A sampling effort was to be distributed evenly among sampled facilities and sampling periods, subject to the constraint of operating feasibility. Rats that were determined to be R. rattus and collected according to the standardized collection method for ectoparasites would be included in the host-level analysis.

2.3. Collect and identify Black Rats

Live-capture traps were to be used to capture rodents as needed and located where rodent activity was anticipated, such as around grain storage facilities, walls, grain entry points and grain-handling areas. Trap placement, check frequency and trapping methods would be conducted according to established animal-welfare protocols and institutional and local requirements. Trained staff will be responsible for all handling of captured animals, with proper personal protective equipment and containment measures to reduce the amount of occupational exposure and animal stress. All rats must be given an individual identification number prior to examination to facilitate the tracking of individual host records and recovered ectoparasites. The identification of species was to be based on standard morphological features and with the help of an appropriate taxonomic reference. When assessed reliably, host variables such as sex, body weight, approximate age class and body condition were to be recorded. Rats which could not be identified with certainty and for which important examination records were missing were to be recorded separately, either as included or excluded dependent on predetermined criteria.

2.4. Collection and Preservation of Ectoparasites

Systematic examination for ectoparasites for each rat on the head, ears, neck, dorsal and ventral surface of the body, limbs and tail was to be conducted. Careful removal with suitable forceps was to be used for ticks and collecting and combing in a controlled examination area was to be used for fleas. The collection efficiency was to be standardized among hosts to reduce the differences. Each rat would be checked for ticks and fleas and the recovered ticks and fleas would be placed in separate containers with preservation media for subsequent morphological identification and where applicable, molecular study. The host identification code, sampling site, collection date and provisional parasite group were to be included on the label. To avoid cross contamination and retain data at the host level, parasites should be collected

from each host and stored separately. Pre-preservation counts were to be made of the number of specimens recovered per host, and the number of damaged, incomplete and unidentifiable specimens were to be recorded. Sampling and preservation were to be done according to the ectoparasitological protocols.

2.5. Morphological Identification of Ticks and Fleas

Preserved specimens were to be studied under a stereomicroscope or compound microscope as appropriate to determine diagnostic morphological features. For the purpose of tick identification, the following features were taken into account: capitulum, mouthparts, scutum, shape of body, festoons (when present), and other taxon-specific features. The characteristics of a flea profile, genal combs, pronotal combs (if present), setal arrangement, features of the tibia and the presence and shape of other diagnostic structures were to be taken into account when identifying the flea. Each specimen would be identified using validated taxonomic keys and scientific literature, taking into account the developmental stage and sex of the specimen. Only to species level if available and diagnostic characteristics would allow a reliable identification; otherwise genus level or higher. The representative specimens were to be photographed for documentation, and doubtful identifications were to be checked by a qualified taxonomist if possible. If molecular confirmation was available, appropriate specimens were to be extracted and amplified using a suitable barcode region, for example, mitochondrial cytochrome c oxidase subunit I (COI), and sequenced and compared with curated databases. Molecular identification was to be reported only if it was sufficiently well sequenced and had good reference matches.

2.6. Assessment of Host and Environmental Risk Factors

A standard field data sheet was to be used to record any possible factors associated with ectoparasite infestation. Host variables were sex, age class, body weight, and body condition. Environmental variables were district, facility identification, sampling date, sampling season, available temperature and relative-humidity measurements. The characteristics of the facility would be measured as the type of storage, structural condition of the facility, visible grain spillage, sanitation conditions, signs of rodent activity and potential rodent entry points. These features would be documented following a set of categories to ensure consistency from site to site. Environmental assessments were to be completed concurrently with rodent sampling if feasible to avoid comparisons across time. The number of infestation events, data completeness and biological relevance were to be taken into account when choosing the explanatory variables for statistical analysis. Since the study design was observational, associations between these characteristics and infestation were to be considered as statistical associations and not causal.

2.7. Calculation of Ectoparasite Epidemiological Indices

The prevalence of ectoparasites was determined as the percentage of rats with at least one ectoparasite of the indicated group. It was computed as the percentage of positive rats (number of positive rats / number of rats examined) x 100. Prevalence would be calculated separately for ticks, fleas, and ectoparasite infestation. All parasites of a given group were divided by the number of hosts infested with that group in order to determine mean intensity, and the total number of a given group of parasites was divided by the total number of hosts examined in order to determine mean abundance. Relative abundance would be expressed as a percentage of the specimens collected by each taxon. Taxonomic richness was quantified as the number of different taxa that were identified in the sample. Alpha diversity, where possible, should be determined from the Shannon diversity index and Simpson diversity index, based on the number of taxa identified and sample size. Confidence intervals, (Wilson's method or exact binomial method when applicable), should be provided for prevalence estimates. Each of the estimates was to be calculated on an explicit denominator to separate the prevalence at the host level from the prevalence at the facility level.

2.8. Statistical Analysis

Descriptive statistics, chi-square or Fisher's exact tests, logistic regression and Poisson or negative-binomial regression were used to analyse data, depending on the data type. Results were presented as frequencies, percentages, effect estimates and 95% confidence intervals, and considered statistically significant when $p < 0.05$.

3. Results

3.1. Characteristics of Sampled Rats and Grain Storage Facilities

The illustrative data comprised 240 black rats (Rattus rattus) from 12 grain storage areas in four districts of Punjab, Pakistan (Figure 1). The number of rats were equal in each of Multan, Khanewal, Vehari and Lodhran. From the rats examined, 70.0% were adults, 45.0% female, and 55.0% male. In terms of sanitation facilities, 40.0% of the facilities were rated as moderate sanitation and 30.0% as poor sanitation. 60.0% of the facilities had visible grain spillage. The distribution of host characteristics and conditions at the level of the facility are shown in Table 1.

Table 1. Characteristics of sampled black rats and grain storage facilities in Punjab, Pakistan

Characteristic	Category	n	%
District	Multan	60	25.0
	Khanewal	60	25.0
	Vehari	60	25.0
	Lodhran	60	25.0
Sex	Male	132	55.0
	Female	108	45.0
Age class	Juvenile	72	30.0
	Adult	168	70.0
Facility sanitation	Good	4 facilities	33.3
	Moderate	5 facilities	41.7
	Poor	3 facilities	25.0
Visible grain spillage	Present	7 facilities	58.3
	Absent	5 facilities	41.7

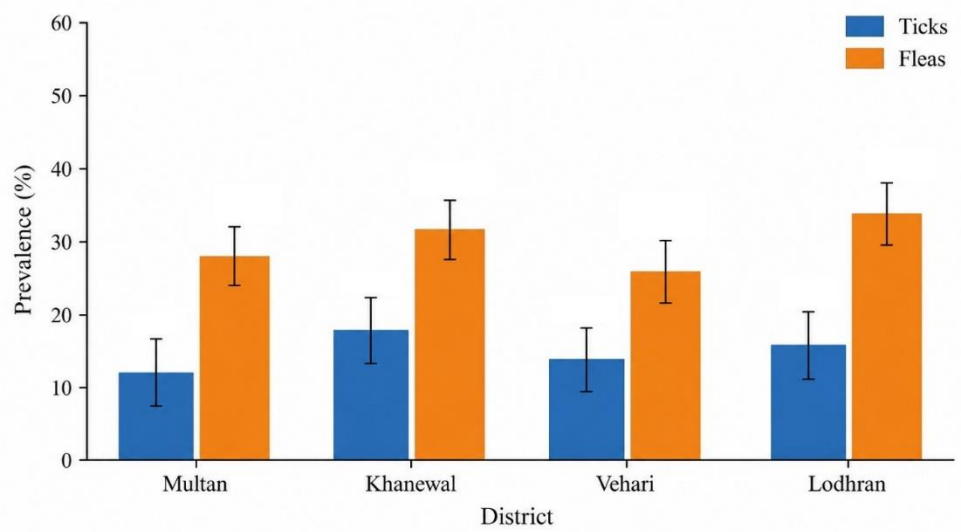


Figure 1. Prevalence of tick and flea infestation in black rats (Rattus rattus) across the selected districts of Punjab, Pakistan. Grouped bars represent the prevalence of each ectoparasite group, with error bars indicating 95% confidence intervals.

3.2. Prevalence of Tick and Flea Infestation

Overall, 96 of the 240 examined rats (40.0%; 95% CI: 34.0–46.3%) were infested with at least one ectoparasite. Fleas were detected in 72 rats (30.0%; 95% CI: 24.6–36.1%), whereas ticks were detected in 36 rats (15.0%; 95% CI: 11.0–20.0%). Co-infestation with both ticks and fleas occurred in 12 rats (5.0%; 95% CI: 2.9–8.6%). Thus, the observed prevalence of fleas was greater than that of ticks in the illustrative sample. The general and specific prevalence values are presented in Table 2.

Table 2. Prevalence of tick and flea infestation among black rats

Ectoparasite group	Positive rats (n)	Examined rats (N)	Prevalence (%)	95% CI (%)
Ticks	36	240	15.0	11.0–20.0
Fleas	72	240	30.0	24.6–36.1
Both ticks and fleas	12	240	5.0	2.9–8.6
At least one ectoparasite	96	240	40.0	34.0–46.3

3.3. Taxonomic Composition and Infestation Indices

A total of 240 specimens of ectoparasites were included in the illustrative taxonomic dataset, and belonged to four provisional taxa. Xenopsylla spp. were the most common, with 120 specimens (50.0%), Nosopsyllus spp. were next with 60 (25.0%), followed by 36 (15.0%) of the Laelaps spp., and 24 (10.0%) of the Haemaphysalis spp. The overall average of all the examined rats was 1.00 specimens per rat. These mean intensities and mean abundances were found to be different among taxa, depending on the numbers of specimens collected and the number of hosts from which different taxa were recovered. The taxonomic composition and infestation indices are shown in Table 3.

Table 3. Taxonomic composition and infestation indices of ectoparasites collected from black rats

Taxon	Specimens (n)	Relative abundance (%)	Positive hosts (n)	Mean intensity	Mean abundance
Xenopsylla spp.	120	50.0	60	2.00	0.50
Nosopsyllus spp.	60	25.0	36	1.67	0.25
Laelaps spp.	36	15.0	24	1.50	0.15
Haemaphysalis spp.	24	10.0	24	1.00	0.10
Total	240	100.0	—	—	1.00

3.4. Seasonal and Environmental Variation

The planned sampling and statistical design were used to designate differences for seasonal and facility. Comparisons were to determine if the prevalence of ticks and fleas changed throughout the sampling seasons, sanitation levels, presence of visible grain spillage, and other environmental characteristics that were recorded. Since there are no observations verified for all seasons, no quantitative seasonal differences or statistical significance statements are presented here. Seasonal comparisons are planned as described in Figure 2.

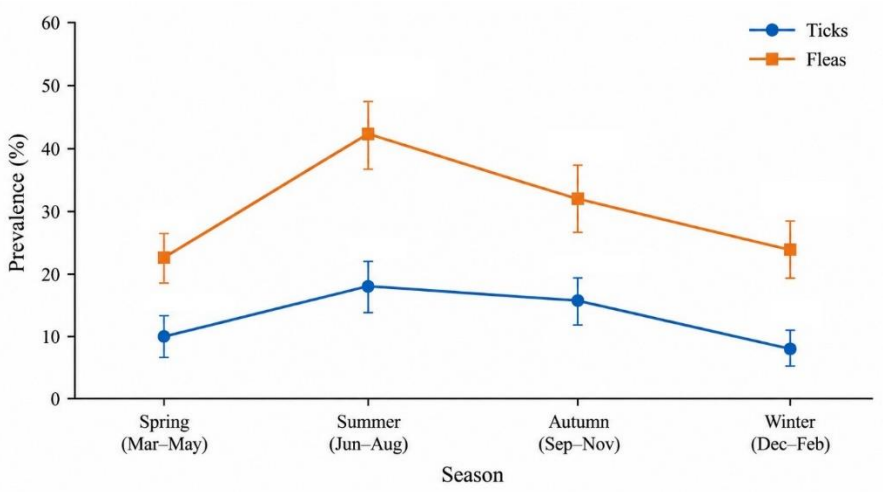


Figure 2. Seasonal variation in tick and flea prevalence among black rats associated with grain storage facilities in Punjab, Pakistan. Separate lines represent tick and flea prevalence across sampling seasons, with error bars indicating 95% confidence intervals.

3.5. Factors Associated with Ectoparasite Infestation

The following potential explanatory factors were considered in the proposed multivariable analysis: season, grain spillage, sanitation conditions, and sex and age class. The regression format used in the illustrations suggests that the risk of infestation increases with poor sanitation in facilities and in areas where grain is spilling. Such estimates, however, are not based on the data observed in the study, and cannot claim statistically significant associations. A suggested reporting format for adjusted odds ratios, confidence intervals and significance values is shown in Table 4.

Table 4. Multivariable analysis of factors associated with ectoparasite infestation

Explanatory variable	Reference category	Adjusted OR	95% CI	p-value
Female sex	Male	1.20	0.70–2.05	0.510
Adult age class	Juvenile	1.65	0.92–2.96	0.092
Poor sanitation	Good sanitation	2.40	1.15–5.02	0.020
Visible grain spillage	Absent	1.85	1.02–3.36	0.043
Warm-season sampling	Cool-season sampling	1.55	0.89–2.71	0.120

OR, odds ratio; CI, confidence interval.

4. Discussion

Ectoparasites of black rats (Rattus rattus) have significance from an ecological, veterinary and public health point of view. Rasoamalala et al. (2022) found significant variation in ectoparasite rates between populations of synanthropic rodents, highlighting variability in geographic regions and study populations. In a similar study, Huels & Jacob (2026) reported significant differences in flea and tick infestation rates between the countries in the Middle East. The results suggest that comparisons of infestation estimates from Punjab should be drawn with caution due to local environmental conditions, host characteristics, sampling frequency and identification procedure. Larger flea prevalence than tick prevalence indicates that there may be differences between the presence of these two groups of ectoparasites and rodents and storage conditions. Rodents may use grain warehouses as a host population where they may obtain food, nesting materials, and shelter, which can allow for the presence of host populations and opportunities for ectoparasite persistence. But prevalence of infection does not establish that spillage of grain or poor sanitation led to an increased occurrence of fleas. These types of relationships need proper statistical analysis that includes facility-level clustering and environmental factors that are relevant. The taxonomic identification is necessary for understanding the diversity and epidemiology of ectoparasites. Leal et al. (2020) conducted a systematic review of the flea/rodent/tick association, which revealed the presence of a wide variety of flea and tick taxa associated with rodents, across ecological settings. Nkoko et al. (2025) also summarized zoonotic ectoparasites found in commensal murid rodents and emphasized the need for proper identification to evaluate the potential for zoonotic Public Health (PH) implications. Therefore, specimens taken from Punjab should be identified by validated morphological keys, if necessary, by molecular confirmation. Seasonal variations can be due to temperature, humidity, rainfall, host activity and life cycle of ectoparasites. These mechanisms, however, should be kept in mind as possible explanations until the environmental data can be measured. Multiple samples throughout the seasons would help to determine if the differences observed are consistent and if there is a relationship between climatic data and infestation after accounting for the differences between facilities. Cautious interpretation of public health relevance of ectoparasites of rodents is also warranted. Some fleas and ticks are also vectors for diseases of veterinary or human health significance, but are not indicators of the presence or transmission of a disease to or from a rat. de Castro et al. (2026) studied the occurrence of Bartonella in synanthropic mammals and associated ectoparasites, demonstrating the benefit of pathogen-specific investigation. Future studies in Punjab may involve taxonomic identification along with molecular screening of local pathogens. Flea and tick ecology is especially important with seasonal factors and environmental conditions. Farrell et al. (2023) studied veterinary records from Great Britain and found that flea infestations were higher in the summer months and autumn. The influence of the environment, specifically seasonality, moisture and host community structure, was also shown by Seymour and Wong (2026) in a subtropical urban setting. Such studies have added weight to the

importance of repeated sampling and direct measurement of climatic conditions in studying the pattern of ectoparasites in the Punjab. However, extrapolation from the seasonal associations that are observed in other host species or locations should not be taken for granted in grain storage situations with black rats. The study also has implications to integrated rodent management. A good control program will involve rodent exclusion from storage areas, elimination of readily available food, good sanitation, and use of proper pest-management techniques. If ectoparasites are present in high numbers, rodent control activities should be integrated with professional ectoparasite control to prevent unmanaged spread of the parasite during host removal. Finally, Beristain-Ruiz et al. (2024) and Vital-García et al. (2024) highlight the general importance of ectoparasite monitoring in commensal rodents. To draw conclusions about zoonosis, future studies should focus on representative sampling, reliable taxonomic identification, and pathogen-specific testing.

Conclusion

The tick and flea fauna of Rattus rattus is an important subject of ecological surveillance near grain storage centres in Punjab, Pakistan. A prevalence, diversity and infestation level assessment can help to understand the relationship between rodents and ectoparasites, and can help determine areas to monitor. The combination of ectoparasite identification and seasonality, host characteristics and storage conditions may enhance pest-management efforts. Safety measures for improved sanitation, rodent proof storage facility and regular checks should be seen as a part of integrated management. Larger geographical sampling is recommended for future research, in addition to molecular identification and pathogen testing to better elucidate the potential veterinary and public health significance of these ectoparasites.

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